

## REMARKS

The Specification has been amended to include sequence identification numbers which were omitted at the time of filing.

Attached hereto is a marked-up version of the changes made to the Specification by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

The undersigned hereby states that the computer readable form copy (CRF copy) of the Sequence Listing and the paper copy of the Sequence Listing, submitted in accordance with 37 C.F.R. § 1.825(a) and (b), respectively, are the same and contain no new matter. Accordingly, entry of the Sequence Listing into the above-captioned case is respectfully requested.

Respectfully submitted,

Dated: 1/30/02

By: Brenda J. Wallach  
Brenda J. Wallach  
Registration No. 45,193

Morrison & Foerster LLP  
3811 Valley Centre Drive  
Suite 500  
San Diego, California 92130-2332  
Telephone: (858) 720-7961  
Facsimile: (858) 720-5125

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the Specification:**

Paragraph beginning at page 95, line 1, has been amended as follows:

The present invention also provides expression vectors in which the recombinant PKS genes of the invention are under the control of a *Myxococcus xanthus* promoter. To construct an illustrative vector, the promoter of the *pilA* gene of *M. xanthus* was isolated as a PCR amplification product. Plasmid pSWU357, which comprises the *pilA* gene promoter and is described in Wu and Kaiser, Dec. 1997, *J. Bact.* 179(24):7748-7758, was mixed with PCR primers Seq1 and Mxpil1 primers:

Seq1: 5'-AGCGGATAACAATTTACACAGGAAACAGC-3' (SEQ ID NO:1); and

Mxpil1: 5'-TTAATTAAGAGAAGGTTGCAACGGGGGGC-3' (SEQ ID NO:2),

and amplified using standard PCR conditions to yield an ~800 bp fragment. This fragment was cleaved with restriction enzyme *KpnI* and ligated to the large *KpnI-EcoRV* restriction fragment of commercially available plasmid pLitmus 28 (New England Biolabs). The resulting circular DNA was designated plasmid pKOS35-71B.

Paragraph beginning at page 96, line 4, has been amended as follows:

The sequence of the *pilA* promoter in these plasmids is shown below (SEQ ID NO:3).

CGACGCAGGTGAAGCTGCTTCGTGTGCTCCAGGAGCGGAAGGTGAAGCCGGTCGGCAG  
CGCCGCGGAGATTCCCTTCCAGGCGCGTGTGTCATCGCGGCAACGAACCGGCGGCTCGAA  
GCCGAAGTAAAGGCCGGACGCTTTCGTGAGGACCTCTTCTACCGGCTCAACGTCATCA  
CGTTGGAGCTGCCTCCACTGCGCGAGCGTTCCGGCGACGTGTCGTTGCTGGCGAACTAC  
TTCCTGTCCAGACTGTCGGAGGAGTTGGGGCGACCCGGTCTGCGTTTCTCCCCCGAGAC  
ACTGGGGCTATTGGAGCGCTATCCCTTCCCAGGCAACGTGCGGCAGCTGCAGAACATG  
GTGGAGCGGGCCGCGACCCTGTCGGATTACAGACCTCCTGGGGCCCTCCACGCTTCCACC  
CGCAGTGCGGGGCGATACAGACCCCGCCGTGCGTCCCGTGGAGGGCAGTGAGCCAGG  
GCTGGTGGCGGGCTTCAACCTGGAGCGGCATCTCGACGACAGCGAGCGGCGCTATCTC

Paragraph beginning at page 147, line 19, has been amended as follows:

Paragraph beginning at page 148, line 22, has been amended as follows:

90-66.1: 5' GCGGG AAGCTT TCACGGCGCAGGCCCTCGTGGG 3' (SEQ ID NO:6)

90-67: 5' GC GGTACC TTCAACAGGCAGGCCGTCTCATG 3' (SEQ ID NO:7)  
 linker KpnI primer

Paragraph beginning at page 149, line 29, has been amended as follows:

The myxothiazol promoter was PCR amplified from *Stigmatella aurantiaca* chromosomal DNA (strain DW4) using primers 111-44.3 and 111-44.5 (shown below). The ~554 bp band was cloned into the *HincII* site of pNEB193 to create pKOS90-107. Plasmid pKOS90-107 was cut with *PstI* and *XbaI* and Klenow filled-in. The 560 bp band was cloned into pKOS90-102 and pKOS90-106 cut with *PacI* and Klenow filled-in (*PacI* cuts only once in pKOS90-102 and pKOS90-106). Plasmids were screened for the correct orientation. The MTA promoter/pKOS90-102 plasmid was named pKOS90-114 (13.36 kb) and MTA promoter/pKOS90-106 plasmid was named pKOS90-113 (13.26 kb).

111-44.3 5' AA AAGCTT AGGCGGTATTGCTTTCGTTGCACT 3' (SEQ ID NO:8)  
 linker HindIII primer

111-44.5 5' GG TTAATTAAGGTCAGCACACGGTCCGTGTGCAT 3' (SEQ ID NO:9)  
 linker PacI primer

Paragraph beginning at page 150, line 22, has been amended as follows:

The putative promoter for TA along with *taA*, which encodes a putative transcriptional anti-terminator, was PCR amplified from strain TA using primers 111-44.8 (AAAGATCTCTCCCGATGCGGGAAGGC) (SEQ ID NO:10) and 111-44.9 (GGGGATCCAATGGAAGGGGATGTCCGCGGAA) (SEQ ID NO:11). The ca. 1.1 kb fragment was cleaved with *BamHI* and *BglII* and ligated into pNEB193 cleaved with *BamHI*. This plasmid is designated pKOS111-56.1. The plasmid pKOS111-56.1 was cut with *EcoRI* and *HindIII* and Klenow filled-in. The ~1.1 kb band was cloned into pKOS90-102 and pKOS90-106 cut with *PacI* and Klenow filled-in (*PacI* cuts only once in pKOS90-102 and pKOS90-106). Plasmids were screened for the correct orientation. The TA promoter/90-102 plasmid was named pKOS90-115 (13.9kb), and the TA promoter/pKOS90-106 plasmid was named pKOS90-111 (13.8kb).

Paragraph beginning at page 152, line 17, has been amended as follows:

These plasmids are electroporated into *Myxococcus* host cells containing the epothilone PKS genes, and kanamycin resistant transformants selected to identify the single crossover recombinants. These transformants are selected for galactose resistance to identify the double crossover recombinants, which are screened by Southern analysis and PCR to identify those containing the desired recombination event. The desired recombinants are grown and tested for epothilone production.

111-44.6 | 5' GG TTAATTAACATCGCGCTATCAGCAGCGCTGAG3' (SEQ ID NO:12)  
linker PacI primer

111-44.7 | 5' GG TTAATTAA TCCTCAGCGGCTGACCCGCTCGCG3' (SEQ ID NO:13)  
linker PacI primer

Paragraph beginning at page 153, line 9, has been amended as follows:

The downstream flanking region of the epothilone PKS gene was PCR amplified using primers 90-103 (5'-AAAAAATGCATCTACCTCGCTCGTGGCGGTT-3') (SEQ ID NO:14) and 90-107.1 (5'-CCCCC TCTAGA ATAGGTCGGCAGCGGTACCCG-3') (SEQ ID NO:15) from plasmid pKOS35-78.2. The ~2 kb PCR product was cut with *NsiI/XbaI* and ligated with pSL1190 digested with *NsiI* and *SpeI* to create pKOS90-123 (~5.4 kb). A ~2 kb PCR fragment amplified with primers 90-105 (5'-TTTTTATGCATGCGGCAGTTTGAACGG-AGATGCT-3') (SEQ ID NO:16) and 90-106 (5'-CCCCCGAATTCTCCCGGAAGGCACACGGAGAC-3') (SEQ ID NO:17) from pKOS35-78.2 DNA was cut with *NsiI* and ligated with pKOS90-123 digested with *NsiI/EcoRV* to create pKOS90-130 (~7.5 kb). When this plasmid is cut with *NsiI*, and the DNA ends made blunt with the Klenow fragment of DNA polymerase I and religated, plasmid pKOS90-131 is created. To clone the *galK/kan<sup>r</sup>* cassette into this plasmid, plasmid KG-2 is cut with *BamHI/NdeI* and made blunt with the Klenow fragment of DNA polymerase I. The 3 kb fragment is cloned into the *DraI* site of pKOS90-131 (*DraI* cuts three times in the vector) to create plasmid pKOS90-132 (10.5 kb). The *NsiI* site is used for the purpose of creating the desired change from cysteine to alanine to effect the KS2 knockout. When pKOS90-130 is cut with *NsiI*, made blunt with the Klenow fragment from

DNA polymerase I and re-ligated, the codon for cysteine is replaced with a codon for alanine. The resulting plasmid can be introduced into *Myxococcus xanthus* strains of the invention in accordance with the protocols described above to create the desired strains.

Paragraph beginning at page 160, line 8, has been amended as follows:

Inactivation of the KR domain in extender moduler 4 of the epothilone PKS results in a hybrid PKS of the invention useful in the production of 13-keto epothilones. The extender module 4 KR domain was modified by replacing the wild-type gene with various deleted versions as described below. First, fragments were amplified using plasmid pKOS39-118B (a subclone of the *epoD* gene from cosmid pKOS35-70.4) as a template. The oligonucleotide primers for forming the left side of the deletion were TL3 and TL4, shown below:

TL3: 5'-ATGAATTCATGATGGCCCGAGCAGCG (SEQ ID NO:18); and

TL4: 5'-ATCTGCAGCCAGTACCGCTGCCGCTGCC (SEQ ID NO:19).

The oligonucleotide primers for forming the right side of the deletion were TL5 and TL6, shown below:

TL5: 5'-GCTCTAGAACCCGGAAGTGGCGTGGCCTGT (SEQ ID NO:20); and

TL6: 5'-GCAGATCTACCGCGTGAGGACACGGCCTT (SEQ ID NO:21).

Paragraph beginning at page 160, line 22, has been amended as follows:

The PCR fragments were cloned into vector Litmus 39 and sequenced to verify that the desired fragments were obtained. Then, the clone containing the TL3/TL4 fragment was digested with restriction enzymes *Pst*I and *Bam*HI, and the ~ 4.6 kb fragment was isolated. The 2.0 kb PCR fragment obtained using primers TL5/TL6 was treated with restriction enzymes *Bgl*II and *Xba*I and then ligated to either (i) the "short" KR linkers TL23 and TL24 (that are annealed together to form a double-stranded linker with single-stranded overhangs) to yield pKOS122-29; or (ii) the "long" (epoDH3\*) linker, obtained by PCR using primers TL33+TL34 and then treatment with restriction enzymes *Nsi*I and *Spe*I, to yield plasmid pKOS122-30. The sequences of these oligonucleotide linkers and primers are shown below:

TL23: 5'-GGCGCCGGCCAAGAGCGCCGCGCCGGTCGGCGGGCCAGCCGGGGACGGGT (SEQ ID NO:22);  
TL24: 5'-CTAGACCCGTCCCCGGCTGGCCCGCCGACCGGCGCGGCGCTCTTGGCCG-GCGCCTGCA (SEQ ID NO:23);  
TL33: 5'-GGATGCA7GCGCCGGCCGAAGGGCTCGGA (SEQ ID NO:24); and  
TL34: 5'-TCACTAGTCAGCGACACCGGCGCTGCGTTT (SEQ ID NO:25).

Paragraph beginning at page 162, line 21, has been amended as follows:

Replacement of the extender module 5 KR, DH, and ER domains of the epothilone PKS with a heterologous KR domain, such as the KR domain from extender module 2 of the rapamycin PKS or extender module 3 of the FK520 PKS, results in a hybrid PKS of the invention useful in the production of 13-hydroxy epothilones. This construction is carried out in a manner similar to that described in part A of this example. The oligonucleotide primers for amplifying the desired portions of the *epoD* gene, using plasmid pKOS39-118B as a template, were:

TL7: 5'-GCGCTCGAGAGCGCGGGTATCGCT (SEQ ID NO:26);  
TL8: 5'-GAGATGCATCCAATGGCGCTCACGCT (SEQ ID NO:27);  
TL9: 5'-GCTCTAGAGCCGCGCGCCTTGGGGCGCT (SEQ ID NO:28); and  
TL10: 5-GCAGATCTTGGGGCGCTGCCTGTGGAA (SEQ ID NO:29).

Paragraph beginning at page 163, line 5, has been amended as follows:

The PCR fragment generated from primers TL7/TL8 was cloned into vector LITMUS 28, and the resulting clone was digested with restriction enzymes *NsiI* and *Bg/II*, and the 5.1 kb fragment was isolated and ligated with the 2.2 kb PCR fragment generated from TL9/TL10 treated with restriction enzymes *Bg/II* and *XbaI* and ligated to the KR cassettes. The KR cassette from the FK520 PKS was generated by PCR using primers TL31 and TL32 and then digestion with restriction enzymes *XbaI* and *PstI*. These primers are shown below:

TL31: 5'-GGCTGCAGACCCAGACCGCGGGCGACGC (SEQ ID NO:30); and  
TL32: 5'-GCTCTAGAGGTGGCGCCGGCCGCCCCGGCG (SEQ ID NO:31).

Paragraph beginning at page 164, line 7, has been amended as follows:

Inactivation of the KR domain of extender module 6 of the epothilone PKS results in a novel PKS of the invention capable of producing the 9-keto-epothilones. The KR domain can be inactivated by site-specific mutagenesis by altering one or more conserved residues. The DNA and amino acid sequence of the KR domain of extender module 6 of the epothilone PKS is shown below:

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36710      36720      36730      36740      36750
GACGGCACCTACCTCGTGACCGGCGGTCTGGGTGGGCTCGGTCTGA
D G T Y L V T G G L G G L G L>
36760      36770      36780      36790      36800
GCGTGGCTGGATGGCTGGCCGAGCAGGGGGCTGGGCATCTGGTGCTGGTG
S V A G W L A E Q G A G H L V L V>
36810      36820      36830      36840      36850
GGCCGCTCCGGTGCGGTGAGCGCGAGCAGACGGCTGTCCGCCGCGCT
G R S G A V S A E Q Q T A V A A L>
36860      36870      36880      36890      36900
CGAGGCGCACGGCGCGCGTGTACGGTAGCGAGGGCAGACGTCGCCGATC
E A H G A R V T V A R A D V A D>
36910      36920      36930      36940      36950
GGGCGCAGATCGAGCGGATCCTCCGCGAGGTTACCGCGTCGGGGATGCCG
R A Q I E R I L R E V T A S G M P>
36960      36970      36980      36990      37000
CTCCGCGGCGTCGTTTCATGCGGCCGGTATCCTGGACGACGGGCTGCTGAT
L R G V V H A A G I L D D G L L M>
37010      37020      37030      37040      37050
GCAGCAAACCCCGCGCGGTTCCGCGCGGTTCATGGCGCCCAAGGTCCGAG
Q Q T P A R F R A V M A P K V R>
37060      37070      37080      37090      37100
GGGCCTTGACCTGCATGCGTTGACACGCGAAGCGCCGCTCTCCTTCTTC
G A L H L H A L T R E A P L S F F>
37110      37120      37130      37140      37150
GTGCTGTACGCTTCGGGAGCAGGGCTCTTGGGCTCGCCGGGCCAGGGCAA
V L Y A S G A G L L G S P G Q G N>
37160      37170      37180      37190      37200
CTACGCCGCGGCCAACACGTTCTCGACGCTCTGGCACACCACCGGAGGG
Y A A A N T F L D A L A H H R R>
37210      37220      37230      37240      37250
CGCAGGGGGCTGCCAGCATTGAGCATCGACTGGGG CTGTTTCGCGGACGTG
A Q G L P A L S I D W G L F A D V>
GGTTTG (SEQ ID NO:32)
G L> (SEQ ID NO:33)

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Paragraph beginning at page 165, line 16, has been amended as follows:

The DNA and amino acid sequence of the mutated and inactive KR domain of extender module 6 of the novel 9-keto-epothilone PKS provided by the present invention is shown below:

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36710      36720      36730      36740      36750
GACGGCACCTACCTCGTGACCGGCGCTCTGGGTGGGCTCGGTCTGA

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D G T Y L V T G A L G G L G L>
  36760      36770      36780      36790      36800
GCGTGGCTGGATGGCTGGCCGAGCAGGGGGCTGGGCATCTGGTGTCTGGTG
S V A G W L A E Q G A G H L V L V>
  36810      36820      36830      36840      36850
GGCCGCTCCGGTGCGGTGAGCGCGGAGCAGACGGCTGTCTGCCGCGCT
G R S G A V S A E Q Q T A V A A L>
  36860      36870      36880      36890      36900
CGAGGCGCACGGCGCGCGTGTCTACGGTAGCGAGGGCAGACGTCTGCCGATC
E A H G A R V T V A R A D V A D>
  36910      36920      36930      36940      36950
GGGCGCAGATCGAGCGGATCCTCCGCGAGGTTACCGCGTCGGGGATGCCG
R A Q I E R I L R E V T A S G M P>
  36960      36970      36980      36990      37000
CTCCGCGGCGTCTTCATGCGGCCGGTATCCTGGACGACGGGCTGTCTGAT
L R G V V H A A G I L D D G L L M>
  37010      37020      37030      37040      37050
GCAGCAAACCCCCGCGCGGTTCCGCGCGGTCATGGCGCCCAAGGTCCGAG
Q Q T P A R F R A V M A P K V R>
  37060      37070      37080      37090      37100
GGGCCTTGACCTGCATGCGTTGACACGGAAGCGCCGCTCTCCTTCTTC
G A L H L H A L T R E A P L S F F>
  37110      37120      37130      37140      37150
GTGCTGTACGCTTCGGGAGCAGGGCTCTTGGGCTCGCCGGGCCAGGGCAA
V L Y A S G A G L L G S P G Q G N>
  37160      37170      37180      37190      37200
CTTCGCCACGGCCAACACGTTCTCGACGCTCTGGCACACCACCGGAGGG
F A T A N T F L D A L A H H R R>
  37210      37220      37230      37240      37250
CGCAGGGGCTGCCAGCATTGAGCATCGACTGGGGCCTGTTTCGCGGACGTG
A Q G L P A L S I D W G L F A D V>
GGTTTG (SEQ ID NO:34)
G L> (SEQ ID NO:35)

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Paragraph beginning at page 168, line 1, has been amended as follows:

A first PCR is used to generate an ~1.6 kb fragment from pKOS39-125 DNA used as template. The PCR fragment is subcloned into vector LITMUS28 at the *Hind*III and *Bgl*II sites and sequenced; a plasmid with the desired sequence is designated P1. The oligonucleotides used in this PCR are:

TLII-1: 5'-ACAAGCTTGCGAAAAAGAACGCGTCT (SEQ ID NO:36) ; and  
 TLII-2: 5'-CGAGATCTGCCGGGCGAGGAAGCGGCCCTG (SEQ ID NO:37).

Paragraph beginning at page 168, line 8, has been amended as follows:

A second PCR is used to generate an ~1.9 kb fragment using pKOS39-125 DNA as template. The PCR fragment is subcloned into vector LITMUS28 at the *Nsi*I and *Spe*I sites and sequenced; a plasmid with the desired sequence is designated P2. The oligonucleotides used in this PCR are:

TLII-3B: 5'-GCATGCATGCGCCGGTCGATGGTGAG ([SEQ ID NO:38](#)); and

TLII-4: 5'-AGACTAGTCACCGGCTGGCCCACCACAAGG ([SEQ ID NO:39](#)).

Paragraph beginning at page 168, line 15, has been amended as follows:

Plasmid P1 is then digested with restriction enzymes *Bgl*II and *Spe*I, and the 4.5 kb fragment is isolated and ligated with the ~1.9 kb *Nsi*I-*Spe*I restriction fragment from plasmid P2 and with one of the three replacement AT fragments (FKAT2, epoAT2, tmbAT3) isolated as *Nsi*I-*Bgl*II restriction fragments to obtain plasmids P3.1, P3.2, and P3.3. The replacement AT fragments are generated by PCR using the following oligonucleotide primers:

for FKAT2:

TLII-20: 5'-GCATGCATCCAGTAGCGGTCACGGCGGA ([SEQ ID NO:40](#)); and

TLII-21: 5'-CGAGATCTGTGTTCGCGTTCCCCGGGCAG ([SEQ ID NO:41](#));

for tmbAT3:

TLII-13: 5'-GCATGCATCCAGTAGCGCTGCCGCTGGAAT ([SEQ ID NO:42](#)); and

TLII-14: 5'-GCAGATCTGTGTTCGTGTTCCCCGGCCA ([SEQ ID NO:43](#)); and

for epoAT2:

TLII-17: 5'-GCATGCATCCAGTACCGCTCGCGCTG ([SEQ ID NO:44](#)); and

TLII-18: 5'-CGAGATCTGTCTTCGTCTTCCCCGGCCAG ([SEQ ID NO:45](#)).

Paragraph beginning at page 170, line 4, has been amended as follows:

A first PCR is used to generate an ~1.8 kb fragment from pKOS39-125 DNA used as template. The PCR fragment is subcloned into vector LITMUS28 at the *Nsi*I and *Spe*I sites and sequenced; a plasmid with the desired sequence is designated P4. The oligonucleotides used in this PCR are:

TLII-5: 5'-GGATGCATGTCTGAGCCTGACGCCCCGCCG ([SEQ ID NO:46](#)); and

TLII-6: 5'-GCACTAGTGATGGCGATCTCGTCATCCGCCGCCAC (SEQ ID NO:47).

Paragraph beginning at page 170, line 11, has been amended as follows:

A second PCR is used to generate an ~2.1 kb fragment using pKOS039-118B DNA as template. The oligonucleotides used in this PCR are:

TL16: ACAGATCTCGGCGCGCTGCCGCCGGAG (SEQ ID NO:48); and

TL15: GGTCTAGACTCGAACGGCTCGCCACCGC (SEQ ID NO:49).

Paragraph beginning at page 170, line 16, has been amended as follows:

The PCR fragment is subcloned into LITMUS 28 at the *EcoRV* restriction site, and a plasmid with the desired sequence is identified by sequencing and designated as plasmid pKOS122-4. Plasmid pKOS122-4 is then digested with restriction enzymes *Bgl*II and *Spe*I, and the 4.8 kb fragment is isolated and ligated with the ~1.8 kb *Nsi*I-*Spe*I restriction fragment from plasmid P4 and with one of the three replacement AT fragments (FKAT3, epoAT5, tmbAT4) isolated as *Nsi*I-*Bgl*II restriction fragments to obtain plasmids P5.1, P5.2, and P5.3. The replacement AT fragments are generated by PCR using the following oligonucleotide primers:

for FKAT3:

TLII-11: 5'-GTATGCATCCAGTAGCGGACCCGCTCGA (SEQ ID NO:50); and

TLII-12: 5'-GCAGATCTGTGTGGCTCTTCTCCGGACA (SEQ ID NO:51);

for tmbAT4:

TLII-15; 5'-GCATGCATCCAGTAGCGCTGCCGCTGGAAC (SEQ ID NO:52); and

TLII-16; 5'-GGAGATCTGCGGTGCTGTTACGGGGCA (SEQ ID NO:53); and

for PCR epoAT5:

TLII-19; 5'-GTAGATCTGCTTTCCTGTTACCCGGACA (SEQ ID NO:54); and

TL8 (see part B of this Example).

Paragraph beginning at page 172, line 6, has been amended as follows:

The PCR fragment generated from primers TL11 and TL12 using plasmid pKOS39-118B as a template is cloned into vector LITMUS 28. The PCR primers used are:

TL11: 5'-GGATGCATCTCACCCCGCGAAGCG (SEQ ID NO:55); and

TL12: 5'-GTACTAGTCAAGGGCGCTGCGGAGG (SEQ ID NO:56).

Paragraph beginning at page 173, line 18, has been amended as follows:

A first PCR is used to generate an ~1.8 kb fragment from pKOS39-124 DNA used as template. The PCR fragment is subcloned into vector LITMUS28 at the *Xba*I and *Bgl*II sites and sequenced; a plasmid with the desired sequence is designated P9. The oligonucleotides used in this PCR are:

TLII-7: 5'-GCAGATCTGCCGCGGAGGAGCTCGCGAT (SEQ ID NO:57); and

TLII-8: 5'-CATCTAGAGCCGCTCCTGTGGAGTCAC (SEQ ID NO:58).

Paragraph beginning at page 174, line 1, has been amended as follows:

A second PCR is used to generate an ~1.9 kb fragment using pKOS39-124 DNA used as template. The PCR fragment is subcloned into vector LITMUS28 at the *Nsi*I and *Spe*I sites and sequenced; a plasmid with the desired sequence is designated P10. The oligonucleotides used in this PCR are:

TLII-9B: 5'-GGATGCATGCGCCGGCCGAAGGGCTCGGAG (SEQ ID NO:59); and

TLII-10: 5'-GCACTAGTGATGGCGATCGGGTCCTCTGTCGC (SEQ ID NO:60).

Paragraph beginning at page 175, line 14, has been amended as follows:

In one embodiment, a strain that produces 10, 11-dehydroepothilone D is constructed by inactivating the enoyl reductase (ER) domain of extender module 5. In one embodiment, the ER inactivation is accomplished by changing the two glycines (-Gly-Gly-) in the NADPH binding region to an alanine and serine (-Ala-Ser-). The 2.5 kb BbvCI-HindIII fragment from plasmid pKOS39-118B (a subclone of the *epoD* gene from cosmid pKOS35-70.4) has been cloned into

pLitmus28 as pTL7 which is used as a template for site directed mutagenesis. The oligonucleotide primers for introducing the –Gly-Gly- to –Ala-Ser- mutations into the NADPH binding domain are:

TLII-22, 5'-TGATCCATGCTGCGGCCGCTAGCGTGGGCATGGCCGC (SEQ ID NO:61)

TLII-23, 5'-GCGGCCATGCCCACGCTAGCGGCCGCAGCATGGATCA (SEQ ID NO:62).